MYCOLOGIA

VOL. XII MAY, 1920 No. 3

THE LIFE HISTORY OF ASCOBOLUS MAGNIFICUS

ORIGIN OF THE ASCOCARP FROM TWO STRAINS

B. O. DODGE

(WITH PLATES 7, 8, AND FIGURES 1–28 IN THE TEXT)

During the years since *Ascobolus magnificus* was first described (6), I have been carrying on from time to time culture studies of this fungus with the hope of being able to settle several puzzling questions that have arisen with regard to it. Apparently it has been collected in Porto Rico several times and some of these specimens have been identified by Dr. F. J. Seaver and deposited in the herbarium of the New York Botanical Garden. The identification of the forms from Porto Rico could be checked up by the use of artificial cultures. It is certainly unknown in Europe or otherwise except from my cultures. In this preliminary paper I shall discuss briefly the following topics: (1) The development of the primordia—ascogonia and antheridia. (2) The asexual or *Papulospora* stage. (3) Intrahyphal mycelium. (4) The necessity of two strains in sexual reproduction.

Atkinson (1), in his usual vigorous style, arrayed the evidence against a theory of the origin of the Ascomycetes from the Florideae and endeavored to show how the Oömycetes, through *Dipodascus,* may have been the ancestors of our "higher" Ascomycetes. It was his opinion that the trichogyne could have arisen by the further development of the receptive papilla of the oögonium. He says that no one has ever proved that the multi-

[MYCOLOGIA for March (12: 59–114) was issued April 8, 1920]

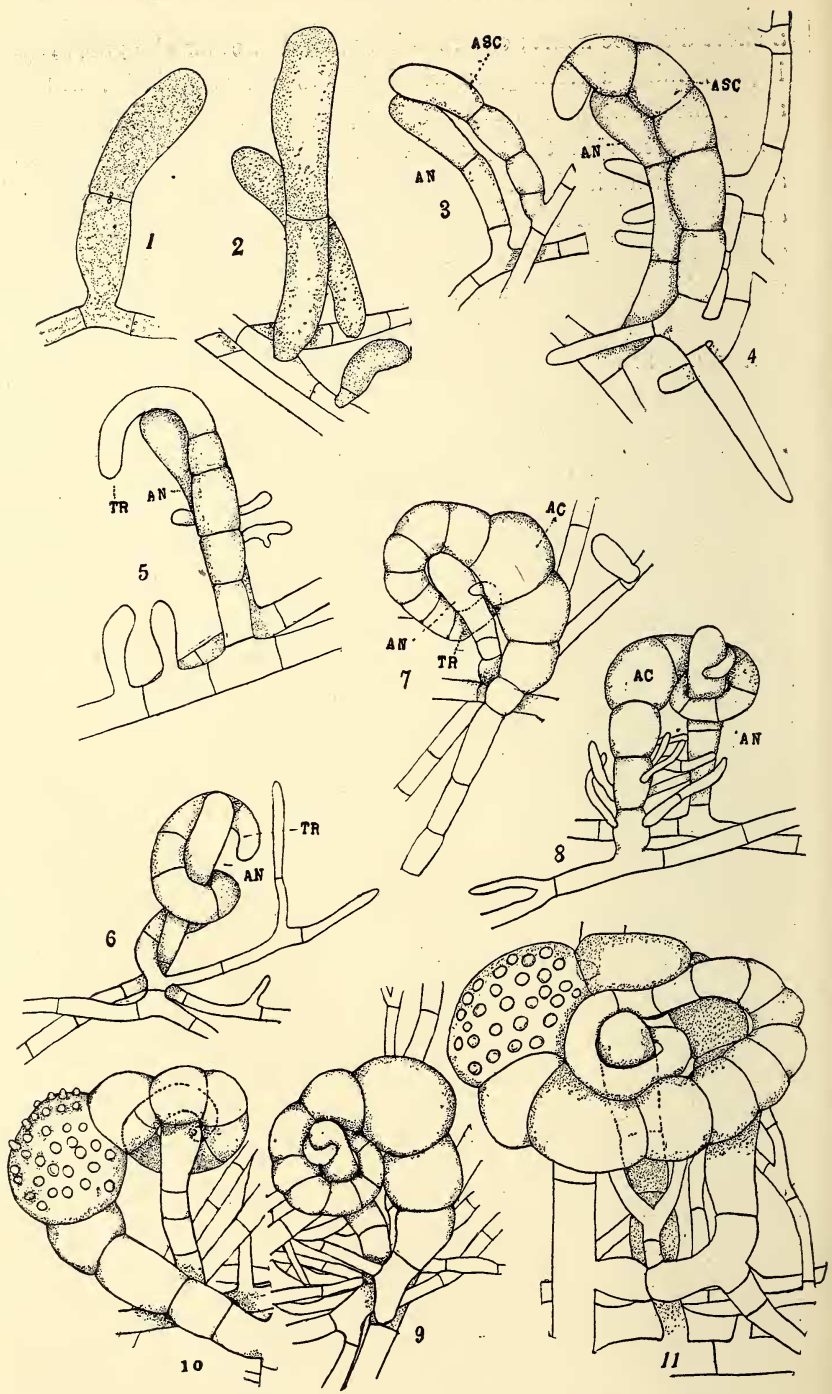
cellular trichogynes of the lichens and other Ascomycetes function in sexual reproduction. These structures have arisen through "progressive sterilization" and are taking on more and more vegetative functions now that sexuality has been lost. About the time Atkinson was formulating this theory, Killian (11) was publishing a preliminary paper on *Venturia* in which he says the nuclei from the antheridium pass into the trichogyne and travel down the long ascogonial coil, the cross walls of which disorganize. The theory of progressive sterilization of the trichogyne is thus overthrown immediately and completely in the event Killian's claim can be confirmed. I have not as yet seen his final paper on the subject (12). In a still later paper on *Cryptomyces* by the same author (13) there are reported other interesting discoveries, all of which, if true, go to show that one should not be too dogmatic in considering a subject such as the sexuality of the Asomycetes about which so little is really known. Little short of a screen demonstration of the passage of the male nuclei from the antheridium into the ascogenous cell will be accepted as final proof that the multiseptate trichogyne functions in sexual reproduction. There are those who deny that in *Pyronema* the simple one-celled trichogyne functions. Brown (2) claims that he studied a strain in which the trichogyne did not fuse with the antheridium at all. Unfortunately he lost this curious strain before his paper appeared in print.

THE ORIGIN OF THE ASCOCARP

In a fertile culture from four to six days old, one can find short one- or two-celled club-shaped branches growing in an erect or oblique position at the surface of the medium. They may be scattered about singly in certain regions (Text fig. 1) or they are more commonly associated in pairs, and sometimes in groups of three or four, all very much alike (Text figs. 2–5). In a very few hours some of the paired branches elongate (Text fig. 4). Both members may be somewhat curved and inclined, one slightly above the other (Text fig. 5). In such case the lower one ceases to elongate and remains a slightly curved two- or three-celled antheridium. Very frequently, however, both structures arise at

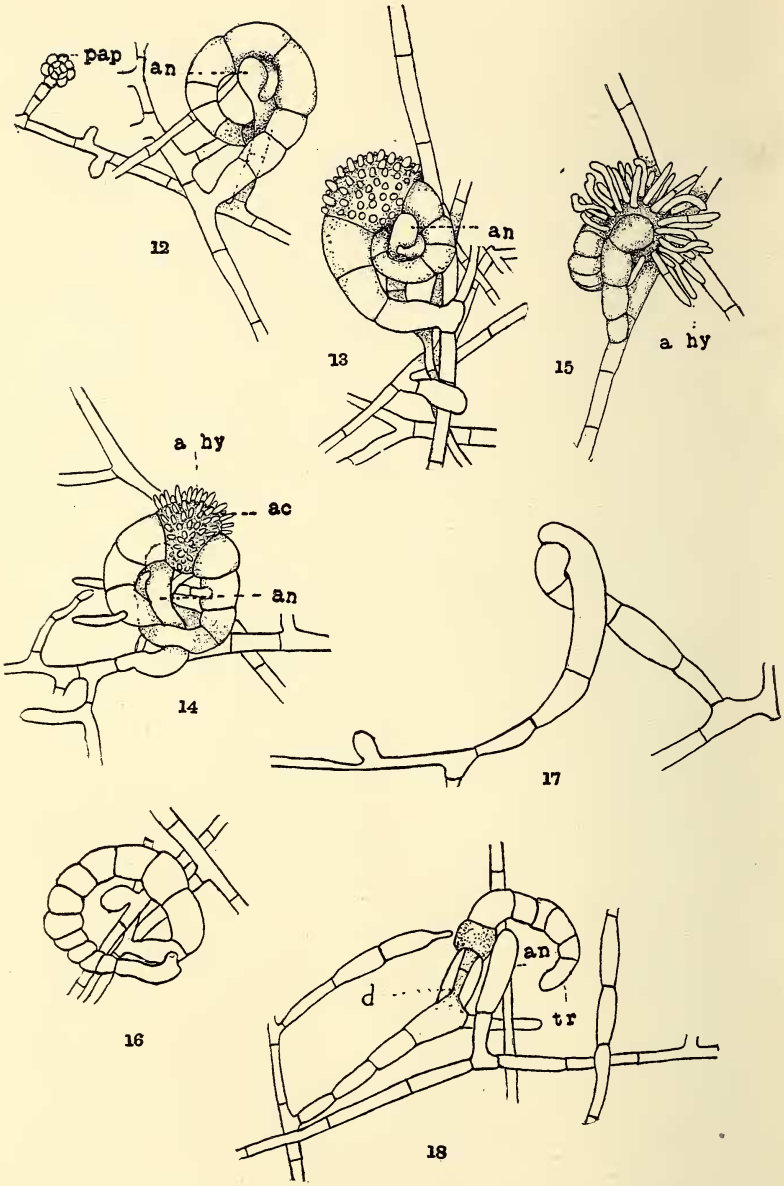
a short distance apart; one remains erect, the other elongates rapidly, forming a trichogyne which grows out and circles widely about the antheridium, drawing it up into the coiled portion, the end of which coils tightly about the end cell of the antheridium and fuses with it (Text figs. 8, 10). The habit of growing erect on the surface of the medium is a hindrance to making photographs or camera lucida drawings of the primordia as they develop. The ascogonia are always knocked over on the antheridium and flattened down when a cover glass is used.

It is not at all difficult to prove in every normal case that this club-shaped antheridium is present. By using a high-powered binocular microscope and a pair of the finest dissecting needles, or needles made of glass drawn out, the antheridium can be pulled out of the trichogyne coil provided fusion has not yet taken place. In Pl. 7, figs. 1 and 3, are shown photographs of a pair of young primordia with different magnifications, and figs. 2 and 4 show the same pairs after the antheridium has been pulled out of the encircling trichogyne coil. No fusion has taken place; the antheridium is plump and the contents are granular. An attempt to separate the organs in later stages, that is, after fusion of the cells, always results in a rupture of the trichogyne, sometimes the break occurring adjacent to the ascogenous cell (Pl. 7, fig. 7). In most cases short hyphal branches soon grow out from the stalk of the ascogonium and wrap about the stalk of the antheridium, so that it is impossible to separate the primordia (Pl. 7, figs. 5, 7, 8 and text fig. 8). This evidently is a further provision for maintaining the erect habit. It seems that there are two phases in the life of this species when an abundance of air is necessary, first at spore germination and second at the origin of the ascocarps. Only those ascospores germinate that lie on the surface of the medium and primordia never form beneath the surface of the agar. *A. Winteri* and *A. carbonarius* are indifferent to the amount of air at both of these periods. The ascogenous cell, which can soon be recognized, begins to enlarge rapidly (Text figs. 7, 8, 9, and Pl. 7, fig. 9) and ascogenous hyphae grow out in considerable numbers sometimes before sterile hyphae have begun to bud out to form the fruit body. (Text figs. 10, 13, 14, 15.)


1
2
ASC
AN
3
ASC
AN
4
TR
AN
5
AC
AN
TR
7
AC
AN
8
TR
AN
6
9
10
11

The form which the ascogonium takes as it develops depends greatly upon the position of the antheridium. Cases are illustrated in Text fig. 6 and in Pl. 7, fig. 6, in which the ascogonium becomes spirally coiled about the antheridium. In the latter figure the end of the trichogyne is plainly visible as it coils about the end of the antheridium. The type shown in Pl. 7, figs. 5, 8, is very common and fig. 10 shows another fairly common type in which the trichogyne makes a wide sweeping coil and the end runs upward along the antheridium (Text figs. 12, 14). Text fig. 15 shows a form in which the ascogenous hyphae have grown out to a considerable length before a single sterile hypha has appeared from the stalk of the ascogonium. The antheridium arises from the hypha crossing beneath from an oblique angle so that it is not shown in the figure. A rather complicated system is shown in Text fig. 11, in which it is difficult to determine the origin of the antheridium, and the trichogyne seems to be unnecessarily long. The antheridium is frequently quite a distance away and it may be that there is an inherent tendency to develop a long trichogyne even in cases where the antheridium grows nearby. In the specimens illustrated in Text fig. 10 the antheridium is really at some distance from the ascogonium, but the preparation was crushed down with a cover glass so that the ascogonium fell over on the antheridium as it always does in mounting the primordia. The camera lucida drawings reproduced in the text were made eight years ago from material preserved for a time in glycerine and then mounted in glycerine jelly. These preparations are very transparent, flattened out and distorted, still the essential features can be made out. Better preparations were made this

TEXT FIGS. 1–11. 1. A single two-celled primordium. 2. A group of three structures, two of which may pair up. 3. A typical pair of primordia, both curved, one lying slightly above the other. 4. The ascogonium arises from the end cell of a hypha and curves over the antheridium. 5. Trichogyne beginning to coil about the antheridium, the ascogenous cell not yet differentiated; sterile hyphae are growing out of the stalks of the primordia. 6. Spiral archicarp. Compare with fig. 6 in Plate 7. 7. Fertilization has taken place; the ascogenous cell is differentiated. 8. Outgrowth of sterile hyphae from the base of the archicarp. 9. The most common type of primordia. 10. Ascogenous hyphae beginning to grow out. These primordia are exceptionally long-stalked. 11. Complicated coil with a long trichogyne.



TEXT FIGS. 12–18. 12. *Papulospora* on the same hyphae with the antheridium. 13. Club-shaped aborted structure near functional primordia. 14. Shows a trichogyne somewhat entangled coiling about the antheridium and the stalk of the ascogonium; ascogenous hyphae well advanced. 15. Long ascogenous hyphae have grown out before the sterile hyphae from the stalk of the

year by mounting primordia directly from Flemming's weak fixative (after washing) into glycerine jelly. The darkening effect of the fixative is an aid in photographing the primordia.

I have previously described the young apothecium (8), pointing out that the hymenium is never covered by a pseudoperidium of sterile cells, such as we find in *A. furfuraceus*. *A. magnificus* is exactly like *Pyronema* in this respect. That an apothecium is "at first closed, then opens," in various ways or that the "hymenium is exposed from the first," these are specific but not necessarily generic or family characters in the Discomycetes.

In any fertile culture there can be found a great number of aborted ascogonia. The trichogyne may sometimes be unable to connect with the antheridium (Text fig. 16), or this structure may not mature sufficiently. In Text fig. 18 a good antheridium is shown to have developed but something evidently prevented fertilization. The paired structures shown in Text fig. 17 did not develop completely. Owing to the large size of apothecia of this species only a relatively small number reach maturity in any one culture, but hundreds of primordia are developed in dung cultures and large numbers of apothecia begin growth without maturing unless the older apothecia are removed or die out. Occasionally I have found that the ascogonium affects a weak union with a club-shaped structure developing on the same hypha, suggesting that rarely both sex organs may arise from the same hypha, and other cases where the trichogyne becomes attached to the stalk cell of the ascogonium (Text fig. 16). All such irregularities or abnormalities appear to come to nothing. In most of my text figures the hypha bearing the antheridium appears to lie below that from which the ascogonium arises. This is not necessarily true for every case, as the fertile hyphae may run parallel to each other or the branch bearing the antheridium may lie uppermost and run at any angle to the other.

ascogonium have made their appearance. The antheridium arises from the hypha crossing at an angle. 16. An aborted archicarp, the trichogyne appears to have become slightly attached to the basal cell of the archicarp. 17. A pair of aborted structures such as are frequently found in cultures. 18. "Durchwachsung," the stalk of the archicarp. A well-developed antheridium is present but the trichogyne did not function for some reason.

In such forms as *A. Winteri* and *A. furfuraceus* fully developed normal ascogonia can be found on hyphae where there is no indication whatever of a structure which in any way resembles an antheridium. I am convinced that these species do not possess morphological antheridia, although fertilization may take place in some other fashion. There can be no question, however, of the necessity for both kinds of sex organs in *A. magnificus*. This cannot be over-emphasized, since many students of the Ascomycetes are inclined to accept the view that sexuality exists in only a few forms like *Pyronema* and the powdery mildews.

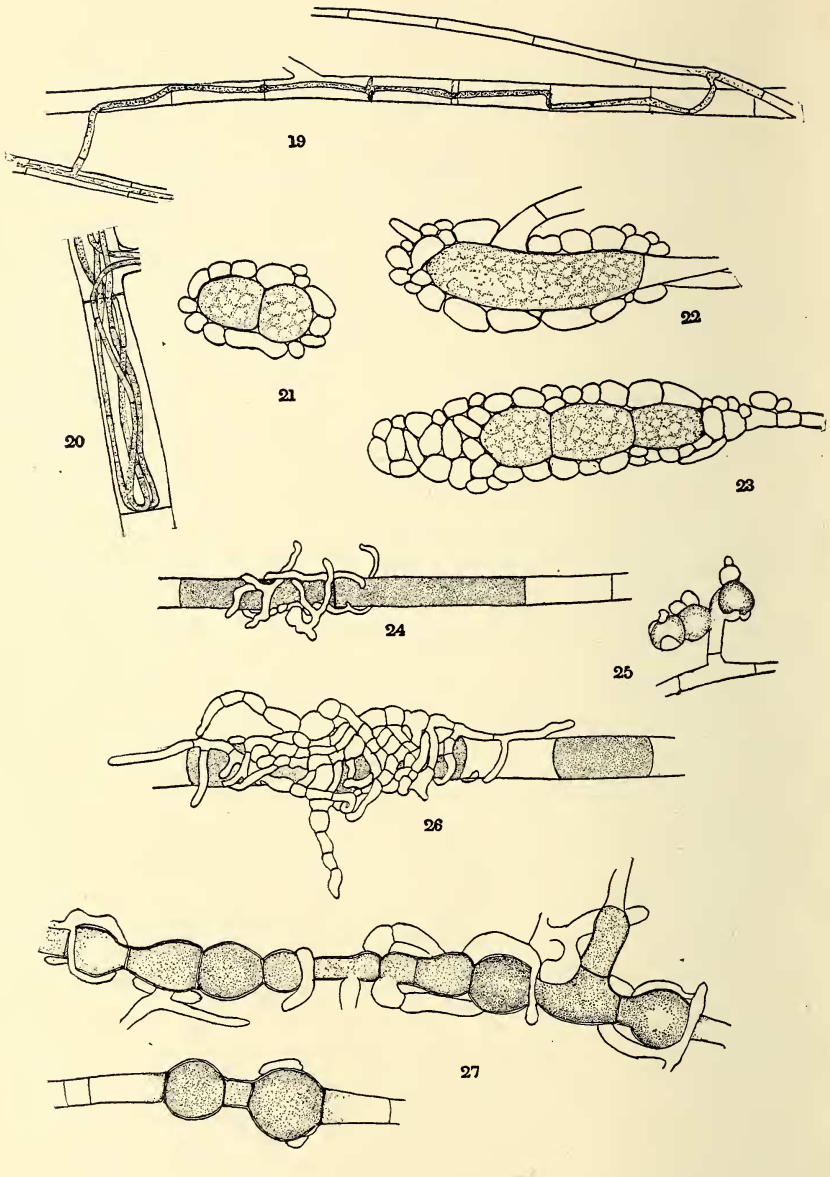
The primordia are visible even without a hand-lens and it is possible to determine within a few hours just when they will first make their appearance in the Petri dish culture. Flemming's weaker fluid is then poured into the dish which can be set aside for examination at leisure. The circle of agar can be floated out into a large battery jar, where it can be washed to remove the fixative previous to mounting the primordia in glycerine jelly. It is much better to study them while they are covered with Flemming's if one is interested in tracing the hyphal connections with them. An oatmeal agar or a potato agar is favorable for the production of large numbers of papulospores or large amounts of mycelium, but the starchy media should not be used if ascocarps are desired. I have dissected a very large number of primordia and have not found a single case where a normal, vigorously growing ascogonium was not accompanied by an antheridium and this is a pretty good indication that there is one species of *Ascobolus* at least in which the sexuality has not been lost or reduced.

We may now turn to the asexual method of reproduction of this species. Many common species of *Ascobolus* in Europe and America are, so far as reported, devoid of asexual spores. *A. parasitica,* described by Van der Wolk (14), is said, on little evidence, to possess a Sclerotium stage as well as a *Rhizostilbella* stage. I have described the curious spore-like bodies that grow in pure cultures of *A. carbonarius* (5). The ascogonia arise directly from certain of these "spores," but further study will be necessary to settle the question as to the exact nature of the others.

PAPULOSPORA MAGNIFICA

A very good illustration of the inadequacy of a classification of fungi based on asexual stages is the genus *Papulospora* as understood by Hotson (3). A true papulospore might be defined as one in which one or two large central storage cells are surrounded by a covering of hyphae which develop from blister-like outgrowths of the storage cell. Hotson has described a number of species and points out that these forms may belong to perfect stages of species in various groups of fungi, that is, *Papulospora* is the bulbil asexual stage liable to be found anywhere among the Ascomycetes.

Papulospores of the type shown in Plate 8, figs. 5, 6, 7, and Text figs. 21, 25, appear in about ten days in all cultures of *A. magnificus* which I have obtained by germinating ascospores. The spores are very hard to germinate, but I have been able to make hundreds of "pure" cultures, many of them one-spore cultures. The papulospores do not germinate readily, either, so that I have made only a few cultures by growing them. I have transferred the mycelium of these one-papulospore strains to several kinds of media without being able to obtain the ascocarps of the *Ascobolus*. At my request Professor J. W. Hoston made an extensive study of the *Papulospora* and published his results describing the species as *P. magnifica* (4). He was unable to obtain ascocarps from the culture which I sent him, and therefore concluded that there was not sufficient evidence to warrant assuming without further proof that it might be an asexual stage of *A. magnificus*. He thought it might occasionally be an intrahyphal parasite as I had once imagined (7). Mr. E. S. Schultz also made a pure one-spore culture of this *Papulospora* for me. I was unable to obtain ascocarps by growing this strain in various media. *Papulospora* stages have been connected with the Melanosporas and there is a very close resemblance between the bulbils of *P. magnifica; P. candida, P. parasitica, P. coprophila* and those of the Melanosporas studied by various investigators.



TEXT FIGS. 19–28. 19. Intrahyphal mycelium in an old culture of *Ascobolus carbonarius*. 20. Intrahyphal mycelium of *A. magnificus*. 21. A papulospore of *A. magnificus* with 2 central cells. 22, 23. Chains of storage cells (papulospores?) surrounded by small hyphae. Such bodies are of frequent occurrence

INTRAHYPHAL MYCELIUM

In old plate cultures it is not difficult to find internal hyphae running back and forth, in and out of the larger hyphae (Pl. 8, fig. 2 and Text fig. 20). Papulospores sometimes arise directly from branches growing out of these internal hyphae. It is only recently that I have been able to prove positively that this is not a case of parasitism of *Papulospora* on *Ascobolus*. On the contrary, it is simply a good example of what has been described under various names, such as Durchwachsungen, cordon interne, accroissement perforant, etc. In old cultures of certain fungi, one can find where bridging hyphae grow out from living cells through dead ones (Pl. 8, figs. 1, 3, 4), and connect up with the next living cells. It is said that a new wall may be formed about the reduced cytoplasm of an old cell, thereby constructing a new hypha of a shorter diameter which occupies only a small portion of the old cell cavity (31). It may happen that for long distances the cells of the hyphae degenerate so that the bud that grows out to bridge the gap must pierce many cross walls (Pl. 8, fig. 2 and Text fig. 19). We thus have what looks like the intrahyphal mycelium of a fungus parasite or a case where a fungus is parasitic on itself. The cross walls of the internal hyphae do not in any way correspond to those of the outer hypha whose septa not infrequently become almost invisible. Sometimes the internal hypha, unable to penetrate the cross wall at the first point of contact, runs along the wall to the other side where it may be able to push through, if not it returns through the same old cell and winds about several times before growing out into the medium. There is usually a swelling on the internal hypha where it passes through a septum of the big cell. De Bary describes *Cicinnobolus* as an intrahyphal parasite of the powdery mildews. Many persons who have investigated the subject of Durchwachsungen have remarked on the close re-

in old cultures on a dung decoction agar. 24, 26. Surface views of heavy-walled brownish cells surrounded by entangling hyphae. 25. Young papulospore that will eventually possess three central storage cells. 27, 28. Chlamydospore-like storage cells, papulospores?.

semblance to parasitism. As Zopf (20) says, "Oft sind entleerte Fäden auf weite Strecken hin von vielfach hin und her gebogenen Keimschläuchen ganz ausgefüllt. Fast möchte man angesichts solcher Bilder glauben, man habe einen fremden Organismus vor sich der als Parasit in den Chaetomienhyphen hause." Text fig. 19 is from an old culture of *A. carbonarius* where there can be no question as to the nature of this internal hypha. Various examples of this phenomenon are reported in the literature and I have arranged a number of the references in the following table:

TABLE I

REFERENCES TO SELF-PENETRATION, DURCHWACHSUNGEN, ETC.

Author	Date	Species
Gasparrini	1856	*Lemna minor*
Schleiden	1872	Saprolegniaceae
Kny & Magnus	1879	Marchantiaceae
Pringsheim	1873	*Saprolegnia*
Kny	1873	*Dasya, Hypnea*
Zopf	1881	*Chaetomium*
Brefeld	1881	*Ascoidea*
Borzi	1885	*Inzengeae*
Holterman	1885	*Ascoidea*
Lindner	1887	*Epicoccum, Alternaria, Botrytis*
Rothert	1892	*Sclerotium hydrophilum*
Klöcker & Schönning	1895–98	*Dematium pullulans*
Ternitz	1900	*Ascophanus carneus*
Duggar & Stewart	1901	*Rhizoctonia*
Beauverie & Guilliermond	1904	*Botrytis cinerea*
Molliard	1904	*Morchella esculenta*
Appel & Bruck	1906	*Sclerotinia libertiana, Botrytis*
Guilliermond	1908	*Gloeosporium nervisequum*
Lewis	1909	*Griffithsia*
Dodge	1912, 1915	*Ascobolus magnificus*
Dodge	1918	*Gymnosporangium*

When the teleutospores of species of *Gymnosporangium* are formed the young spore buds often grow into and through the old buffer cells above (36). If the intrahyphal mycelium found in the powdery mildews is that of a fungus parasitic on another fungus, then it is the only case of the kind known. All of the other examples of this phenomenon have been proved on further investigation to be due to self-penetration, "Durchwachsungen," "d'accroissement perforant," or self-parasitism, so to speak.

Compare Neger's figures of *Melanospora marchica, Papulospora* stage (15) with my figures, Pl. 8, also compare the "groups of chlamydospore-like structures" of *M. papillata* (Hotson, Pl. 2, fig. 7) with my text figures 22 and 23; and further, the "*Acremoniella* type" of spore of *M. cervicula* (Hotson, Pl. 3, fig. 17) with those shown in text figs. 27, 28, and one is struck with the close parallelism between the asexual reproductive bodies of *Melanospora* and those of *Ascobolus magnificus* and the spore balls of *Urocystis* type. As noted we have been unable to obtain ascocarps from one-spore cultures of *P. magnifica.* Furthermore, I have no record of obtaining ascocarps from a one-ascospore culture of *A. magnificus.*

TWO STRAINS NECESSARY FOR SEXUAL REPRODUCTION

I have stated in connection with the description of the primordia that the ascogonia and antheridia ordinarily (or perhaps always) arise from different hyphae. This fact early suggested the reason for the sterility of one-spore cultures. If one sows ascospores in a dung culture he may obtain ascocarps. If they are sowed in an agar medium under proper conditions germination may take place rarely, and I have frequently obtained ascocarps from such cultures, but I have noticed that when one or two transfers of mycelium are made from these fertile cultures the subcultures are apt to be sterile. This is especially true where the ends of the hyphae are cut off and transferred from young cultures. I have planted a single spore on agar in petri dishes and on sterile dung in jelly glasses in several hundred cases without obtaining a single germination, so rarely do the ascospores germinate. On August 16, 1919, I planted ascospores on agar in each of ten petri dishes, heating the cultures at 60° C. for twenty minutes. After twenty-four hours no germination had taken place. On September 2, mycelium was found in seven of the dishes and papulospores were present in each case, but there were no primordia or ascocarps. Transfers of mycelium were made to tube and plate cultures and on September 4 sterilized horse manure in jelly glasses was inoculated with the strains numbered 1, 2, 3, 4 and 5. Strain 6 was lost and strain 7 was

not used. Strain 2 was planted alone and in combination with strains 1, 3, 4 and 5, with the result that in ten days numerous ascocarps appeared on the cultures containing strains 2 + 1 and 2 + 4, while in the cultures containing strains 2, 2 + 3 and 2 + 5,

TABLE II

SUMMARY OF CULTURES OF *Ascobolus magnificus* FROM VARIOUS COMBINATIONS OF STRAINS—1, 2, 3, 4, 5, AND 7

Medium	No. of Cultures	Strains Used	Results	
			Positive	Negative
Sterilized fresh horse manure in jelly glasses and milk bottles	26	2	0	26
	7	1	0	7
	3	3	0	3
	21	4	0	21
	3	5	0	3
	5	7	0	5
	7	2+1	6	1
	2	4+1	0	2
	3	2+2	0	3
	2	2+3	0	2
	22	2+4	21	1
	2	2+5	0	2
	3	2+7	2	1
	3	3+4	3	0
	2	4+4	0	2
	3	5+7	3	0
Horse dung decoction agar in Petri dishes	3	1+1	0	3
	3	1+2	3	0
	1	1+4	0	1
	1	1+5	1	0
	1	1+7	0	1
	36	2+2	0	36
	1	2+3	0	1
	52	2+4	52	0
	5	2+7	5	0
	1	3+3	0	1
	2	3+4	2	0
	40	4+4	0	40
	3	4+7	0	3
	5	5+4	5	9
Horse dung decoction agar in 12-in. test tubes	28	2	0	28
	5	2+2	0	5
	26	4	0	26
	3	4+4	0	3
	20	2+4	19	1
	5	1	0	5
	3	3	0	3
	6	5	0	6
	5	7	0	5

[1] Results are positive when ascocarps appear in cultures, and negative when no ascocarps appear within a month.

there were no fruit bodies although papulospores could be found in all of the cultures. The last three cultures have not produced ascocarps. The following table shows cultures of which a record was kept in testing out the strains. Many others were made simply to obtain primordia for purposes of study.

It is clear that at least for these strains each is sterile by itself or strains 2, 3 and 5 are sterile (in the combinations tested) when placed together, as are strains 1, 4 and 7, but a combination of any one of either group with any one of the other group produces a fertile culture. I have since obtained ten new strains from germinated ascospores. These strains fall into two groups, two of which are like the original No. 2 and eight are like No. 4.

The use of sterilized fresh horse manure in jelly glasses or in milk bottles is preferable when it is simply desired to ascertain whether single strains ever become fertile because this is the natural substratum and the ascocarps reach a large size whenever the culture is fertile. On the other hand, when the fertility of two strains in combination is to be tested one of the transfers may not grow and it is then impossible to draw any conclusions whatever from negative results. Whenever papulospores are found it is certain that one strain has developed a mycelium, while the other may not have grown at all. On the other hand, plate cultures obviate this difficulty since it can be seen (within 24 hours) whether either strain has begun to grow, and if one has not a reinoculation can be made. No single strain culture on dung is reported sterile in the above table where an examination was not made to find papulospores, thus proving that the inoculation was successful. Twelve-inch test tubes containing the dung decoction agar are most satisfactory for keeping pure cultures for a long time. Ascocarps up to a centimeter in diameter have been grown in these large tubes, while I have not been very successful in growing ascocarps in small test tubes.

Not all of the possible combinations of strains 1, 2, 3, 4, 5 and 7 have been tried in these different ways, because the first thing desired was a combination that was sure to produce primordia for purposes of study. Strains 2 and 4 were chosen for the most extensive tests, and it can be said that each is sterile by itself and

fertile in combination with the other after many transfers. The first sub-cultures of strains 2 and 1 on dung in jelly glasses remained sterile from October 10 to January 6, but produced ascocarps within five days when both strains were grown together on dung in a milk bottle.

BEHAVIOR OF MYCELIA IN CULTURE

If a petri dish culture is inoculated on opposite sides with the same strain (4 + 4), the mycelia grow out at the same rate from both sides until they meet along a straight line through the center. There is a narrow region between the two mycelia which remains comparatively free from hyphae as though there was an antagonism or repulsion between the two. Now, if opposite strains such as 2 and 4 are planted in the same culture, the mycelia grow out at about the same rate, meeting near the center of the culture where there appears to be, for a brief period a slight antagonism, at least the rate of growth is much reduced, then the hyphae from either side can be found growing freely across the line of meeting, making a zone plainly visible across the center composed of hyphae from both strains. Numbers of ascogonia and antheridia soon make their appearance, not necessarily at all in a line across the center, such as one finds in the cultures of *Rhizopus*. The hyphae grow so rapidly that both strains are soon found throughout the culture and ascocarps appear in any region whatever. The largest numbers of sex organs, however, generally appear first near the line of meeting of the mycelia and one or two cm. from the periphery.

In view of Blakeslee's discoveries of plus and minus strains in the Zygomycetes and his theories regarding their sexuality, and Burger's recent report on the "pseudo-heterothallic" condition in *Cunninghamella* (15a), it is of importance to determine the question of the sexuality of the strains in this *Ascobolus*. Each of the strains so far isolated is self-sterile, and no sex organs are produced in a one-strain culture. Ascogonia and antheridia ordinarily arise from different hyphal branches. How universal this rule is, I am not prepared to say. I have not seen any good evidence of fertilization between structures arising

side by side on the same hyphal branch. The hyphae of this species are large and they can be traced for long distances in a transparent dung-decoction agar. Anastomoses occur frequently in fertile cultures near primordia, but the question of the sexuality of the strains will not be a difficult one to determine.

Shear (9) has studied species of the genus *Glomerella* and made large numbers of one-spore cultures from ascospores and conidia. He finds that many such strains are fertile in themselves, while others are sterile. He has grown various strains and cultures together without obtaining evidence of what might be called plus and minus strains. Edgerton (10), however, states that he has repeatedly isolated plus and minus strains of *Glomerella* from one-spore cultures. One strain when planted alone produces some perithecia as will the other when it is grown by itself, but when both plus and minus strains are grown together there is a dense black line of perithecia formed where the strains meet. There are, however, perithecia scattered about elsewhere in the culture. Edgerton was unable to find structures corresponding to oögonia and antheridia; however, he offers a theory to account for the behavior of his strains.

No sex organs are developed in the strains of *Ascobolus magnificus* mentioned above except under the contact or chemical stimuli of two strains in the same culture. Are archicarps formed on one strain and antheridia on another? Will each strain remain self sterile indefinitely? Are strains segregated at the time of ascospore formation? Are there neutral strains or pseudo-heterothallic strains? All of these questions remain interesting subjects for further investigation.

SUMMARY

1. The ascocarp of *Ascobolus magnificus* originates from a pair of morphologically distinct primordia—a large ascogonium the end of which functions as a trichogyne, and a club-shaped antheridium.

2. *Papulospora magnifica* Hotson is an asexual stage of *Ascobolus magnificus* Dodge.

3. The intrahyphal mycelium found in old cultures is simply a case of "Durchwachsungen," or "cordon interne."

4. The strains here reported, which were obtained from germinated papulospores or ascospores, were self-sterile in the experiments conducted, but always produced papulospores.

5. Sexual reproduction occurs in cultures containing two strains properly chosen.

COLUMBIA UNIVERSITY,
NEW YORK CITY.

LITERATURE CITED

1. **Atkinson, G. F.** Phylogeny and relationships in the Ascomycetes. Ann. Missouri Bot. Gard. 2: 315–376. *f. 1–10.* Ap 1915.
2. **Brown, W. H.** The development of *Pyronema confluens* var. *inigneum.* Am. Jour. Bot. 2: 289–298. Je 1915.
3. **Hotson, J. W.** Culture studies of fungi producing bulbils and similar propagative bodies. Proc. Am. Acad. Arts & Sci. 48: 227–306. *pl. 1–12.* O 1912.
4. **Hotson, J. W.** Notes on bulbiferous fungi with a key to described species. Bot. Gaz. 64: 265–284. *pl. 21–23.* O 1917.
5. **Dodge, B. O.** Methods of culture and the morphology of the archicarp in certain species of the Ascobolaceae. Bull. Torrey Club 39: 139–197. *pl. 10–15 + f. 1, 2.* 17 My 1912.
6. **Dodge, B. O.** Artificial cultures of *Ascobolus* and *Aleuria.* Mycologia 4: 218–222. *pl. 72, 73.* Jl 1912.
7. **Dodge, B. O.** The *Papulospora* question as related to *Ascobolus.* Science II 41: 4. 29 Ja 1915.
8. **Dodge, B. O.** The morphological relationship of the Florideae and the Ascomycetes. Bull. Torrey Club 41: 157–202 *f. 1–13.* 22 Ap 1914.
9. **Shear, C. L., & Wood, A. K.** Studies of fungous parasites belonging to the genus *Glomerella.* U. S. Dept. Agr. B. P. I. Bull. 252: 1–110. *pl. 1–18 + f. 1–4.* 25 Ja 1913.
10. **Edgerton, C. W.** Plus and minus strains in the genus *Glomerella.* Am. Jour. Bot. 1: 244–254. *pl. 22, 23 + f. 1.* 1914.
11. **Killian, K.** Ueber die Entwicklung der Perithecien bei *Venturia inaequalis* (Cooke). Ad. Ber. Deuts. Bot. Gesells. 33: 164–168. *f. 1, 2.* Ap 1915.
12. **Killian, K.** Ueber die Sexualität der *Venturia inaequalis.* Zeits. Bot. 9: 534– . 1917.
13. **Killian, K.** Morphologie, Biologie und Entwicklungsgeschichte von *Cryptomyces Pteridis* (Rebent.) Rehm. Zeits. Bot. 10: 49–126. *f. 1–31.* 1918.
14. **Van der Wolk, P. C.** *Rhizostilbella rubra* (n. gen. n. spec.) a by-fruit form of *Ascobolus parasiticus* (nov. spec.) and its connection with the "sclerotium disease" of certain tropical cultivated plants (*Sclerotium omnivorum* n. spec.). Myc. Centralb. 4: 236–241. *f. 1–11.* Je 1914.
15. **Neger, F. W.** Über *Urocystis* Ähnliche nebenfruchtorcmen von Hypocreaseen. Myc. Centralb. 4: 273–378. 1914.

15a. **Burger, O. F.** Sexuality in *Cunninghamella.* Bot. Gaz. 68: 134–146. **Au 1919.**

Durchwachsungen, Cordon interne, Self-penetration

16. **Gasparrini, G.** Recherche sulla natura e la escrezione della radici ed osservazioni morphologiche sopra taluni organi della *Lemna minor*. 1–152. *pl. 1–11.* Napoli. 1856.
17. **Schleiden, M. J.** Grundzüge der wissenschatflichen Botanik 1: 264. 1872.
18. **Pringsheim, M.** Weitere Nachträge zur Morphologie und Systematik der Saprolegnieen. Jarb. Wiss. Bot. 9: 196. *pl. 18. f. 9, 10.* 1873.
19. **Kny, L.** Ueber die Bedeutung der Florideen in morphologische und histologische Beziehung. Bot. Zeit. 31: 433. 1873.
20. **Zopf, W.** Zur Entwicklungsgeschichte der Ascomyceten. *Chaetomium.* Nova Acta 42: 199–292. *pl. 14–20.* 1881.
21. **Borzi, A.** *Inzengaea,* ein neuer Askomycet. Jarb. Wiss. Bot. 16: 450–463. *pl. 19, 20.* 1885.
22. **Lindner, P.** Ueber Durchwachsungen an Pilz Mycelium. Ber. Deuts. Bot. Gesells. 5: 153–161. 1887.
23. **Rothert, W.** Ueber *Sclerotium hydrophilum* Sacc. einen sporenlosen Pilz. Bot. Zeit. 50: 321. 1892.
24. **Holterman, C.** Mycologische Untersuchungen aus den Tropen. 1895.
25. **Weleminsky, F.** Ueber Sporenbildungen bei *Dematium pullulans.* Centralb. Bakt. 2 Abt. 5: 297–303. *f. 1–9.* 1899.
26. **Klöcker, A., & Schönning, H.** Uber Durchwachsungen und abnorme Konidien bildungen bei *Dematium pullulans* De Bary und anderen Pilzen. Centralb. Bakt. 2 Abt. 5: 505–507. 1899. See also 1: 777. 1895; 2: 185. 1896; 4: 460. 1898.
27. **Schönning, H., & Klöcker, A.** Phénomenes d'accroisement perforant de *Dematium pullulans* et autres Champignons. C. R. des travaux du laboratoirre de Carlsberg. 5: —. 1900.
28. **Ternetz, C.** Protoplasms bewegung und Fruchtkörperbildung bei *Ascophanus carneus* Pers. Jarb. Wiss. Bot. 35: 273–312. *pl. 7.* 1900.
29. **Duggar, B. M., & Stewart, F. C.** The sterile fungus *Rhizoctonia* as a cause of plant diseases in America. N. Y. Agr. Exp. Sta. Bull. 186: 1–30. *f. 1–23.* Ja 1901.
30. **Lafar.** Handbuch der Technischen Mykologie 1: 170. *f. 29;* 2: 355. *f. 28 (4, 5);* 4: 277. *f. 83 (a–f).* 1904–1907.
31. **Beauverie, J., & Guilliermond, A.** Étude sur la structure du *Botrytis cinerea.* Centralb. Bakt. 2 Abt. 10: 275–381. *f. 1–5;* 311–320. *f. 6–14.* 1904.
32. **Molliard, M.** Forme conidienne et sclérotes de *Morchella esculenta.* Rev. Gén. Bot. 16: 210–218. *pl. 16.* 1904.
33. **Appel, O., & Bruck, F. W.** Arbeiten a. d. k. Biol. Anstalt. f. Land. u. Forstwirtschaft. 5: 189. 1906. See Lafar 2: 355. *f. 28.*
34. **Guilliermond, A.** Recherches sur le développement du *Gloeosporium nervisequum* (*Gnomonia veneta*) et sur so prétendu. transformation en levures. Rev. Gen. Bot. 20: 385. *pl. 15–33* + *f. 1–10.* 1908.
35. **Lewis, I.** The life history of *Griffithsia Bornetianus.* Ann. Bot. 23: 653. *pl. 50. f. 48, 50.* 1909.
36. **Dodge, B. O.** Studies in the genus *Gymnosporangium*—III. The Origin of the Teleutospore. Mycologia 10: 182–193, *pl. 9–11.* Jl 1918.

EXPLANATION OF PLATES

PLATE 7

Fig. 1. Primordia before fertilization, trichogyne not fully developed.

Fig. 2. The same pair of primordia after the antheridium had been pulled out of the trichogyne coil.

Figs. 3 and 4. Same as Figs. 1 and 2 except less highly magnified. The coiled portion of the ascogonium (trichogyne?) shows more distinctly in this position than in Fig. 2.

Figs. 5 and 8. Common types of primordia in which the antheridium is circled by sterile hyphae from the stalk of the ascogonium.

Fig. 6. Spiral type in which the trichogyne is plainly visible coiling about the end of the antheridium.

Fig. 7. Archicarp broken in two in an attempt to pull the antheridium away from the trichogyne after fusion had taken place.

Fig. 9. Short-stalked archicarp, the antheridium not visible.

Fig. 10. Same type as shown in Fig. 9 except that the antheridium is plainly visible.

PLATE 8

Fig. 1. Hyphae bridging a dead cell and connecting two living cells.

Fig. 2. Intrahyphal mycelium resembling the mycelium of a fungus parasite.

Figs. 3 and 4. Clearly the "Durchwachsung" type of bridging hyphae.

Figs. 5 and 6. Papulospores of *Ascobolus magnificus*. At the right in Fig. 5 can be seen a few cells of a hypha of large diameter. Note the small hyphae from which the papulospores arise. This is not always the case as these spores frequently arise from hyphae of large diameter.

Fig. 7. Papulospore arising from an "internal hypha."